ANTIGLUCONEOGENIC ACTIVITY OF GONADAL STEROID ANALOGUES IN RELATION TO LIVER GLUCOCORTICOID–RECEPTOR BINDING

M. K. AGARWAL and F. COUPRY

Inserm U-36, 17 Rue du Fer-à-Moulin, Paris 75005, France

Received 8 August 1977

1. Introduction

Gonadal steroids have been studied intensively over the past decades both for their clinical value in contraception and to delineate the mechanism of steroid hormone action in the appropriate target tissue [1,2]. Although most of their physiological effects are restricted to the respective organ in the male and the female, various sex steroids have been known to alter several hepatic responses [1,2] and to bind to high affinity receptors in liver supernatant fractions [3]. Antiglucocorticoid activity of testosterone in the hepatoma cell cultures [4] and of progesterone in isolated thymocytes [5] has also been documented although such is not the case in vivo [5]. The present report adds a new parameter in the range of metabolic changes inflicted by gonadal steroids in the liver, by exhibiting antiglucocorticoid activity in vivo, and reveals that this may proceed without concurrent antagonism in glucocorticoid–receptor association.

2. Materials and methods

Male, Wistar rats (200–250 g) were bilaterally adrenalectomized under ether anaesthesia and maintained on 1% NaCl ad libitum. Animals were injected intraperitoneally 3–5 days post-surgery with the test steroid suspended in water and sacrificed 4 h later. Livers were quickly removed, weighed and homogenized with a Teflon pestle in 5% trichloroacetic acid (TCA) containing 0.1% silver sulphate. After heating in a boiling water bath for 15 min, 2 ml of the clear supernate was mixed with 6 ml 36 N H_2SO_4. Samples were thoroughly vortexed, heated in a boiling water bath for 6 min, cooled in an ice bath, and quantitated 520 nm in a spectrophotometer against a blank containing only TCA–silver sulphate in place of liver homogenate. A standard curve was run with each experiment consisting of 25, 50 and 100 μg glucose in the initial buffer. The glycogen levels are expressed as μg 5-hydroxymethylfurfural/100 mg fresh liver tissue, as previously described [6,7].

For binding studies, livers were perfused with the incubation buffer (0.01 M Tris–HCl, pH 7.4), homogenized with a Teflon pestle, centrifuged at low speed (20 000 × g, 20 min) followed by ultracentrifugation 105 000 × g, 60 min). The cell sap thus obtained (0.5 ml) was dispensed into Pyrex tubes containing 3×10^{-6} mol/ml nonradioactive competitor of choice. After incubation (60 min, at 3°C), 0.5 ml carbon (Sigma C-5260) suspension (100 mg/ml Tris buffer) was added to each tube. Samples were vortexed, allowed to stand for 10 min and finally centrifuged (5000 × g, 5 min). All manipulations were carried out at 2–4°C. Aliquots, 200 μl, of the reaction mixture were put into 10 ml Unisolve (Kochlight, England) and counted in a Packard Tricarb Scintillation Spectrometer equipped with background substraction and external standardization.

All steroids were purchased from Steroloids (NY). 11-Methoxy ethyl derivative was kindly provided by Drs D. Philibert and J. P. Raynaud, Roussel UCLAF, (Romainville, France). [1,2,4-^{3}H]Triamcinolone acetonide (16 Ci/mM; batch ZT 1423) was obtained from Schwarz Mann (N.J.). All other chemicals were Reagent grade from Merck.

North-Holland Publishing Company – Amsterdam

3. Results

Data in table 1 show that all analogues of both testosterone and estradiol significantly decreased liver glycogen levels as compared to the water-treated control. A synthetic methoxy derivative of estradiol appeared to be the most potent compound in this regard ($P < .001$) although no efforts were made to quantitate the differences between the various materials employed. Relative potency was, however, indicated as follows. Liver glycogen levels were significantly higher when triamcinolone acetonide (TA) was given alone or with gonadal steroid derivative. TA induction of liver glycogen was clearly impaired in presence of a 20-fold excess of either sex hormone analogue in the order

methoxyethyl estradiol > dihydrotestosterone > 6-dehydrotestosterone = 1-dehydrotestosterone > 17β-estradiol > 17α-estradiol > 17β-testosterone.

It is however difficult to say whether these results are in favour of an actual repression of the operon controlling glycogen synthesis or simply an expression of an increase in energy demand than can be met with by the endogenous rate of synthesis.

4. Discussion

Whatever the mechanism, this is a clear demonstration of an antiglucocorticoid action in vivo. Although there is much discussion in the literature on the interaction between sex steroids and carbohydrate reserves, the results are at variance due primarily to different experimental designs and techniques [1,2], and are in contrast with decreased glycogen levels clearly evident here. The implications of this finding in clinical prescription of oestro–progestative therapy are therefore clear.

Association of a steroid with its specific receptor is said to constitute the first step whereby an active hormone initiates tissue-specific processes [8]. It follows that an antagonist would inhibit this binding, as had previously been observed in in vitro systems [4]. Data in table 2 show that binding of [^{3}H]TA to its hepatic receptor was almost totally abolished in presence of 100-fold excess of cold TA. The methoxy derivative of estradiol diminished [^{3}H]TA binding only slightly followed by even smaller decreases with 17β-estradiol. Although the former was most potent both in reversing gluconeogenesis (table 1), and in antagonizing [^{3}H]TA–receptor binding (table 2), the evidence indicates that gonadal steroid analogues reversed gluconeogenesis at a time when they did not significantly diminish [^{3}H]TA binding to its receptor. In view of the multiplicity of steroid hormone vectors [9–12] it is conceivable too that the antagonists could associate themselves with inhibitor proteins physically distinct from the active receptor. On the other hand, high-

Table 1
Antigluconeogenic effect of gonadal steroid analogues

Test compound[a]	Liver glycogen (mg%)	
	–TA	+TA[b]
Control	9.03 ± 0.85	18.6 ± 3.5
Estradiol (17β)	2.12 ± 0.55	9.42 ± 1.19
Estradiol (17α)	1.09 ± 0.06	11.8 ± 0.75
11-Methoxyethyl estradiol	1.00 ± 0.05	2.52 ± 0.38
Testosterone (17β)	2.37 ± 0.31	13.4 ± 1.1
1-Dehydrotestosterone	1.64 ± 0.62	7.69 ± 0.97
6-Dehydrotestosterone	2.50 ± 0.26	7.48 ± 1.49
Dihydrotestosterone	1.66 ± 0.14	5.65 ± 1.21

[a] All materials were given as a single intraperitoneal injection of 20 mg in 1 ml water, 4 h prior to glycogen assay

[b] Triamcinolone acetonide (1 mg) was given with the test compound as above. Each value is the mean ± SE of 4–5 separate determinations

Table 2
Effect of sex steroid analogues on the binding of a synthetic glucocorticoid to its cytoplasmic receptor

Competitor (3×10^{-6} mol/ml)	Binding of 3×10^{-8} [^{3}H]triamcinolone acetonide (cpm/mg protein)[a]
None	2535
Triamcinolone acetonide	124
Estradiol (17β)	1794
Estradiol (17α)	2372
11-Methoxyethyl estradiol	987
Testosterone (17β)	2372
1-Dehydrotestosterone	2344
6-Dehydrotestosterone	2402
Dihydrotestosterone	2443

[a] Each value is a mean of three, separate determinations. Bound radioactivity obtained in presence of 1000-fold excess of nonradioactive TA was substracted from all values to account for non specific binding

affinity binding sites for gonadal hormones was confirmed in parallel studies and has been published earlier [3]. The repressive action may proceed by acceptor occupancy at sites distinct from those that are filled with the glucocorticoid–receptor complex or by actual diminution in the transfer of receptor–bound TA to the appropriate nuclear regions. Further studies are needed to distinguish between these various possibilities.

Acknowledgements

Supported in part by DGRST (75.7.0744), INSERM (CL 76.5.001.4) and CNRS (A1 03 1917). Thanks are due to M. Philippe and L. Berger for technical assistance and to Miss Rebecca Horrell for preparing the typescript.

References

[1] Brotherton, J. (1977) in: Sex Hormone Pharmacology, Academic Press, NY.
[2] Solhanick, H. A., Kipnis, D. M., Vauder Wiele, R. E. eds (1969) Metabolic Effects of Gonadal Hormones and Contraceptive Steroids, Plenum Press, NY.
[3] Eisenfeld, A. J., Aten, R., Weinberger, M., Haselbacher, G., Halpern, K. and Krakoff, L. (1975) Science 191, 862–864.
[4] Samuels, H. H. and Tomkins, G. M. (1970) J. Mol. Biol. 53, 57–74.
[5] Disarbo, D., Rosen, F., McPortland, R. P. and Milholland, R. J. (1977) Ann. NY Acad. Sci. in press.
[6] Agarwal, M. K. (1974) Biochem. Pharmacol. 23, 2577–2584.
[7] Kemp, A. and Kits van Heijningen, J. M. (1954) Biochem. J. 56, 646–648.
[8] Gorski, H. and Ganon, F. (1976) Ann. Rev. Physiol. 38, 425–450.
[9] Agarwal, M. K. (1975) Nature 254, 623–625.
[10] Agarwal, M. K. (1976) FEBS Lett. 67, 260–263.
[11] Agarwal, M. K. (1976) Biochem. Biophys. Res. Commun. 73, 767–772.
[12] Agarwal, M. K. ed (1977) Multiplicity of Steroid Hormone Receptors, Devol. Endocrinol. Vol. 1, Elsevier North-Holland Biomedical Press, Amsterdam.